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Reference	Country
	28-JUN-2000
Agent	Short title
SLK 00 06 29 29. JUN. 2000 SLK	
Action	Term
	—

Patent- og Varemærkestyrelsen
Helgeshøj Allé 81
2630 Tåstrup

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Enzyme Business Patents

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A/S Reg. No. 16201

Deres ref: PA 2000 00989
Vor ref: 10055.000-DK, SLK/SuRH

Dansk patentansøgning Nr. PA 2000 00989

Vi skal herved meddele Dem, at vi ønsker at tilbagetage ovennævnte ansøgning, som blev indleveret den 26-JUN-2000.

De bedes venligst bekræfte, at tilbagetagelsen har fundet sted ved at returnere vedlagte kopi af dette brev i underskrevet stand.

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Enzyme Business Patents

Sten L. Knudsen

Sten Lottrup Knudsen

Dato: _____ Modtaget af: _____

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Patent- og Varemærkestyrelsen

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183 106

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Dato: _____ Modtaget af: _____

Cc: Journal

183106

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Reference	Country
Agent: SLK 00 06 27 SLK — 27. JUN. 2000	Short title
Action	Term: Jeto 00 06 27

Vores ref: PA 2000 00989/DOK2/SPI
Deres ref: 10055.000-DK

Dato: 26. juni 2000

Noted

Novo Nordisk A/S

Vedrørende ansøgning om Field of the Invention .

De har den 26. juni 2000 indleveret en ansøgning om patent.

Vi har givet ansøgningen nr. PA 2000 00989. Vi beder Dem om at oplyse dette nummer ved henvendelse til os om denne sag.

Hvis De ikke har indbetalt ansøgningsgebyret endnu, vil vi senere sende et girokort på de gebyrer, som skal betales for behandlingen af ansøgningen. Vi gør Dem opmærksom på, at disse gebyrer ikke er momspligtige.

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Læs venligst vejledningen til de enkelte punkter

2. Ansøgers fuldmægtigs referencenr.
10055.000-DK

3. International indleveringsdag: ☐ Kapitel I
Internationalt ansøgningsnr.: ☐ Kapitel II

4. Ansøger (fulde navn og adresse): ☐ Flere ansøgere på bagsiden
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5. Fuldmægtig (navn og adresse): Brev- og fakturamodtager:
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6. Opfinder (fornavn, efternavn, adresse):

6a. Opfinder (fornavn, efternavn, adresse): ☐ Flere opfindere på bagsiden

7. Opfindelsens benævnelse:

8. Prioritetspåstand(e): ☐ Flere prioritetspåstande på bagsiden

Dato	Land	Nr.
Dato	Land	Nr.
Dato	Land	Nr.

9. ☒ Ansøgningen omfatter deponering af en prøve af biologisk materiale, som angivet i patentlovens § 8a, stk. 1.

10. ☒ Ansøgningen omfatter en sekvensliste.

11. ☐ Ansøgningen er fremkommet ved deling eller udskillelse.
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13. ☐ Ansøgningen er tidligere indleveret pr. telefax den:

14. Dato og underskrift: 26-06-2000
Sten Lottrup Knudsen
Novo Nordisk A/S

1.1-jan00/ts

1. Gebyrer:
☐ Ansøgningsgebyr
☐ Kravgebyr
☐ ITS-nyhedsundersøgelsesgebyr

12. Bilagsfortegnelse:
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☐ dansk beskrivelse i 1 ekspl.
☐ sammendrag i 1 ekspl.
☐ tegninger i 1 ekspl.
☐ prioritetsdokument
☐ fuldmagt
☐ overdragelsesdokument
☐
☐
Fig nr. _____ ønskes publiceret sammen med sammendraget.

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NZAS-0022989

LIPOLYTIC ENZYME

FIELD OF THE INVENTION

The present invention relates to a lipolytic enzyme, methods of using and producing it, as well as a nucleic acid sequence encoding it.

BACKGROUND OF THE INVENTION

Lipolytic enzymes (such as lipases and phospholipases) are capable of hydrolyzing carboxylic ester bonds in a substrate to release carboxylic acids. They are known to be useful, e.g., in baking and detergents.

A lipase/phospholipase from *Fusarium oxysporum* and its sequence are known. WO 98/26057.

SUMMARY OF THE INVENTION

The inventors have isolated a lipolytic enzyme from *Fusarium sulphureum*. The inventors also isolated the gene encoding the novel lipolytic enzyme and cloned it into an *E. coli* strain.

Accordingly, the invention provides a lipolytic enzyme which may be a polypeptide having an amino acid sequence as the mature peptide shown in SEQ ID NO: 1.

Further, the lipolytic enzyme of the invention may be a polypeptide encoded by the lipolytic enzyme encoding part of the DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 13539.

The lipolytic enzyme may also be an analogue of the polypeptide defined above which:

i) has at least 85 % homology with said polypeptide,

ii) is immunologically reactive with an antibody raised against said polypeptide in purified form,

iii) is an allelic variant of said polypeptide,

Finally, the lipolytic enzyme of the invention may be a polypeptide which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence of SEQ ID NO: 1 encoding the mature polypeptide or a subsequence thereof having at least 100 nucleotides.

The nucleic acid sequence of the invention may comprise a nucleic acid sequence which encodes the lipolytic enzyme described above, or it may encode a lipolytic enzyme and comprise:

a) the DNA sequence encoding a mature lipolytic enzyme cloned into a plasmid present in *Escherichia coli* DSM 13539,

b) the DNA sequence encoding a mature lipolytic enzyme shown in SEQ ID NO: 1, or

c) an analogue of the DNA sequence defined in a) or b) which

i) has at least 80 % homology with said DNA sequence, or

ii) hybridizes at high stringency with said DNA sequence, its complementary strand or a subsequence thereof.

Other aspects of the invention provide a recombinant expression vector comprising the DNA sequence, and a cell transformed with the DNA sequence or the recombinant expression vector.

A comparison with full-length prior-art sequences shows that the mature amino acid sequence of the invention has 82 % homology with the lipase/phospholipase from *Fusarium oxysporum* described above, and the corresponding DNA sequence of the invention shows 77 % homology with that of the *F. oxysporum* enzyme.

DETAILED DESCRIPTION OF THE INVENTION

Genomic DNA source

A lipolytic enzyme of the invention may be derived from a strain of *Fusarium*, particularly *F. sulphureum*, using probes designed on the basis of the DNA sequences in this specification.

A strain of *Escherichia coli* containing a gene encoding lipolytic enzyme was deposited by the inventors under the terms of the Budapest Treaty with the DSMZ - Deutshe Sammmlung von Microorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig DE, Germany. The deposit date was15 June 2000, and the accession number was DSM 13539.

Properties of lipolytic enzyme

The lipolytic enzyme is able to hydrolyze carboxylic ester bonds and is classified as EC 3.1.1 according to Enzyme Nomenclature 1992, Academic Press, Inc. The enzyme has lipase (triacylglycerol lipase) activity (EC 3.1.1.3) and may also have phospholipase activity.

Recombinant expression vector

The expression vector of the invention typically includes control sequences encoding a promoter, operator, ribosome binding site, translation initiation signal,

and, optionally, a selectable marker, a transcription terminator, a repressor gene or various activator genes. The vector may be an autonomously replicating vector, or it may be integrated into the host cell genome.

Production by cultivation of transformant

The lipolytic enzyme of the invention may be produced by transforming a suitable host cell with a DNA sequence encoding the lipolytic enzyme, cultivating the transformed organism under conditions permitting the production of the enzyme, and recovering the enzyme from the culture.

The host organism is preferably a eukaryotic cell, in particular a fungal cell, such as a yeast cell or a filamentous fungal cell, e.g. a strain of *Aspergillus*, *Fusarium*, *Trichoderma* or *Saccharomyces*, particularly *A. niger*, *A. oryzae*, *F. graminearum*, *F. sambucinum*, *F. cerealis* or *S. cerevisiae*. The production of the lipolytic enzyme in such host organisms may be done by the general methods described in EP 238,023 (Novo Nordisk), WO 96/00787 (Novo Nordisk) or EP 244,234 (Alko).

Hybridization

The hybridization is used to indicate that a given DNA sequence is analogous to a nucleotide probe corresponding to a DNA sequence of the invention. The hybridization conditions are described in detail below.

Suitable conditions for determining hybridization between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC (standard saline citrate) for 10 min, and prehybridization of the filter in a solution of 5 x SSC (Sambrook et al. 1989), 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) *Anal. Biochem.* 132:6-13), ^{32}P-dCTP-labeled (specific activity > 1 x 10^9 cpm/µg) probe for 12 hours at approx. 45°C. The filter is then washed two times for 30 minutes in 2 x SSC, 0.5 % SDS at a temperature of at least 55°C, more preferably at least 60°C, more preferably at least 65°C, even more preferably at least 70°C, especially at least 75°C.

Molecules to which the oligonucleotide probe hybridizes under these conditions are detected using a x-ray film.

Alignment and homology

The lipolytic enzyme and the nucleotide sequence of the invention may have homologies to the disclosed sequences of at least 85 %, particularly at least 90 % or at least 95 %, e.g. at least 98 %.

For purposes of the present invention, alignments of sequences and calculation of homology scores were done using a Needleman-Wunsch alignment (i.e. global alignment), useful for both protein and DNA alignments. The default scoring matrices BLOSUM50 and the identity matrix are used for protein and DNA alignments respectively. The penalty for the first residue in a gap is -12 for proteins and -16 for DNA, while the penalty for additional residues in a gap is -2 for proteins and -4 for DNA. Alignment is from the FASTA package version v20u6 (W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448, and W. R. Pearson (1990) "Rapid and Sensitive Sequence Comparison with FASTP and FASTA", Methods in Enzymology, 183:63-98).

Lipase activity (LU)

A substrate for lipase is prepared an emulsion of 5 % by volume of tributyrin (glycerin tributyrate) using 0.1 % gum Arabic as emulsifier. The hydrolysis of tributyrin at 30 °C at pH 7 is followed in a pH-stat titration experiment. One unit of lipase activity (1 LU) equals the amount of enzyme capable of releasing 1 µmol butyric acid/min at the standard conditions. 1 KLU = 1000 LU.

Use of lipolytic enzyme

The lipolytic enzyme of the invention can be used in various industrial application of lipolytic enzymes, e.g. in baking, detergents, diglyceride synthesis (EP 307154), acidolysis, interesterification (WO 8802775), ester hydrolysis, oil degumming (JP-A 2-153997, US 5264367), production of lysolecithin (JP patent 2794574, JP-B 6-087751) and in the process described in PCT/DK 00/00109.

Use in baking

The lipolytic enzyme of the invention can be used in the preparation of dough, bread and cakes, e.g. to improve the elasticity of the bread or cake. Thus, the lipolytic enzyme can be used in a process for making bread, comprising adding the lipolytic enzyme to the ingredients of a dough, kneading the dough and baking the dough to make the bread. This can be done in analogy with WO 9404035 and EP 585988.

Use in detergent

The variant may be used as a detergent additive, e.g. at a concentration (expressed as pure enzyme protein) of 0.001-10 (e.g. 0.01-1) mg per gram of detergent or 0.001-100 (e.g. 0.01-10) mg per liter of wash liquor.

The detergent composition of the invention may for example be formulated as a hand or machine laundry detergent composition including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations. In a laundry detergent, the variant may be effective for the removal of fatty stains, for whiteness maintenance and for dingy cleanup. A laundry detergent composition may be formulated as described in WO 97/04079, WO 97/07202, WO 97/41212, PCT/DK WO 98/08939 and WO 97/43375.

The detergent composition of the invention may particularly be formulated for hand or machine dishwashing operations. e.g. as described in GB 2,247,025 (Unilever) or WO 99/01531 (Procter & Gamble). In a dishwashing composition, the variant may be effective for removal of greasy/oily stains, for prevention of the staining /discoloration of the dishware and plastic components of the dishwasher by highly colored components and the avoidance of lime soap deposits on the dishware.

MATERIALS AND METHODS

Methods

Unless otherwise stated, DNA manipulations and transformations were performed using standard methods of molecular biology as described in Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. et al. (eds.) "Current protocols in Molecular Biology", John Wiley and Sons, 1995; Harwood, C. R., and Cutting, S. M. (eds.) "Molecular Biological Methods for Bacillus". John Wiley and Sons, 1990.

Enzymes

Enzymes for DNA manipulations (*e.g.* restriction endonucleases, ligases etc.) are obtainable from New England Biolabs, Inc. and were used according to the manufacturer's instructions.

Plasmids/vectors

pT7Blue (Invitrogen, Netherlands)

pCaHj483 is described in WO 9704079 and WO 9942566.

Cloning

LA PCR™ in vitro Cloning Kit (TaKaRa) was used for cloning and was used according to the manufacturer's instructions.

Microbial strains

E. coli JM109 (TOYOBO, Japan)

A. oryzae BECh-2 is described in Danish patent application PA 1999 01726. It is a mutant of JaL 228 (described in WO 98/12300) which is a mutant of IFO 4177.

Media and reagents

Cove: 342.3 g/L Sucrose, 20 ml/L COVE salt solution, 10mM Acetamide, 30 g/L noble agar.

Cove-2: 30 g/L Sucrose, 20 ml/L COVE salt solution, 10mM, Acetamide, 30 g/L noble agar.

Cove salt solution: per liter 26 g KCl, 26 g MgSO4-7aq, 76 g KH2PO4, 50ml Cove trace metals.

Cove trace metals: per liter 0.04 g NaB4O7-10aq, 0.4 g CuSO4-5aq, 1.2 g FeSO4-7aq, 0.7 g MnSO4-aq, 0.7 g Na2MoO2-2aq, 0.7 g ZnSO4-7aq.

AMG trace metals: per liter 14.3 g ZnSO4-7aq, 2.5 g CuSO4-5aq, 0.5 g NiCl2, 13.8 g FeSO4, 8.5 g MnSO4, 3.0 g citric acid.

YPG: 4 g/L Yeast extract, 1 g/L KH2PO4, 0.5 g/L MgSO4-7aq, 5 g/L Glucose, pH 6.0.

STC: 0.8 M Sorbitol, 25 mM Tris pH 8, 25 mM CaCl2.

STPC: 40 % PEG4000 in STC buffer.

Cove top agarose: 342.3 g/L Sucrose, 20 ml/L COVE salt solution, 10mM Acetamide, 10 g/L low melt agarose.

MS-9: per liter 30 g soybean powder, 20 g glycerol, pH 6.0.

MDU-pH5: per liter 45 g maltose-1aq, 7 g yeast extract, 12 g KH2PO4, 1 g MgSO4-7aq, 2 g K2SO4, 0.5 ml AMG trace metal solution and 25 g 2-morpholinoethanesulfonic acid, pH 5.0.

EXAMPLES

Example 1: Cloning and expression of lipase gene from *Fusarium sulphureum*

Transformation in Aspergillus strain

Aspergillus oryzae strain BECh-2 was inoculated to 100 ml of YPG medium and incubated for16 hrs at 32°C at 120 rpm. Pellets were collected and washed with 0.6 M KCl, and resuspended 20 ml 0.6 M KCl containing a commercial β-glucanase

product (Glucanex, product of Novo Nordisk A/S) at the concentration of 30 µl/ml. Cultures were incubated at 32°C at 60 rpm until protoplasts formed, then washed with STC buffer twice. The protoplasts were counted with a hematometer and resuspended in an 8:2:0.1 solution of STC:STPC:DMSO to a final concentration of 2.5x10e7 protoplasts/ml. About 3 µg of DNA was added to 100 µl of protoplasts solution, mixed gently and incubated on ice for 30 min. One ml of SPTC was added and incubated 30 min at 37°C. After the addition of 10 ml of 50°C Cove top agarose, the reaction was poured onto Cove agar plate. Transformation plates were incubated at 32°C for 5 days.

PCR screening of lipase

A strain of *Fusarium sulphureum* was used as a genomic DNA supplier.

PCR reactions on *Fusarium sulphureum* genomic DNA was done with two following primer sets: lip3 / lip15 and lip10 / lip17 designed based upon the alignment of 3 lipases from *Fusarium*.

lip3: 5'-carcayggigcigcigcitaytg-3'

lip15: 5'-ccicciariswrtgiccigt-3'

lip10: 5'-ggitgyggigticayiiiggitt-3'

lip17: 5'-ggrtcityiscrtkigtiac-3'

Reaction components (2.6 ng /µl of genomic DNA, 250 mM dNTP each, primer 250 nM each, 0.1 U/ µl of Taq polymerase in 1X buffer (Roche Diagnostics, Japan)) were mixed and submitted for PCR under the following conditions.

Step	Temperature	Time
1	94°C	1 min
2	50°C	1 min
3	72°C	2 min
4	72°C	10 min
5	4°C	forever

Steps 1 to 3 were repeated 30 times.

450bp of fragment and 280 bp of fragment were amplified from primer sets of lip3/lip15 and lip10/lip17, respectively. They were gel-purified with GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) and ligated into a pT7Blue vector with ligation high (TOYOBO, Japan) . The ligation mixtures were transformed into *E. coli* JM109. The resultant plasmids, pT27-0315 and pT27-1017, were sequenced and compared to the *Fusarium oxysporum* lipase, showing that a clone encodes the internal part of the lipase.

Cloning of lipase gene

In order to clone the missing part of the lipase gene, adaptor PCR was done. A cassette was made by mixing of adaptor L and adaptor S.

adaptor L: 5'-ctaatacgactcactatagggctcgagcggccgcccgggcaggt=3'

adaptor S: 5'-acctgccc-3'

3' and 5' of adaptor S are dephosphorylated and amidation, respectively.

1.3 μg of Eco RV digested genome was ligated to the cassette and it was used as a PCR template. Reaction components (7 ng /μl of genomic DNA ligated to cassette, 250 mM dNTP each, primer 250 nM each, 0.05 U/ μl of Expand high fidelity polymerase in 1X buffer (Roche Diagnostics, Japan)) were mixed and submitted for PCR under the following conditions.

Step	Temperature	Time
1	94°C	2 min
2	94°C	10sec
3	55°C	30sec
4	68°C	45sec
step 2-4 repeat 10 times		
5	94°C	10sec
6	55°C	30sec
7	68°C	45sec +20sec/cycle
step 5-7, repeat 20 times		
8	68°C	7min
7	4°C	forever

500 bp of DNA fragment corresponding to N-terimal region was obtained with 27N1long primer and 200 bp of DNA fragment corresponding to C-terminal region was obtained with 27C1long primer.

27N1long: 5'-tggacaaccgttccttgcgca-3'

27C1long: 5'-tacacgtacggtgctcctcgagtgg-3'

Obtained fragments were purified by GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) and sequenced with each primers which amplified the fragment.

The missing C-terminal part was cloned with LA PCR™ in vitro Cloning Kit (TaKaRa) following to the manufacturer's instructions. 350 bp of DNA fragment corresponding to C-terminal region was obtained from Xho I digested genome ligated to Sal I cassette of the kit with 27C2 primer.

27C2: 5'-tatctggcggcggtggcgac-3'

Obtained fragments were purified by GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) and sequenced with 27C2 primer.

The fidelity of taq polymerase is not good so in order to get the right sequence whole gene was amplified the following primers.

27N(Bam): 5'-cgcggatccatgctcctcctaccactcctctcagcc-3'

27C(Sal): 5'-acgcgtcgacttatgatgaacgattcttatggctatccacatactcct-3'

Reaction components (6 ng /μl of genomic DNA, 250 mM dNTP each, primer 250 nM each, 0.05 U/ μl of Expand high fidelity polymerase in 1X buffer (Roche Diagnostics, Japan)) were mixed and submitted for PCR under the following conditions.

Step	Temperature	Time
1	94°C	2 min
2	94°C	10sec
3	55°C	30sec
4	68°C	45sec
step 2-4 repeat 10 times		
5	94°C	10sec
6	55°C	30sec
7	68°C	45sec +20sec/cycle
step 5-7, repeat 20 times		
8	68°C	7min
7	4°C	forever

Amplified DNA fragment was gel-purified with GFX™ PCR DNA and Gel Band Purification kit (amersham pharmacia biotech) and ligated into a pT7Blue vector with ligation high (TOYOBO, Japan). The ligation mixtures were transformed into *E. coli* JM109. Four plasmids, pT27w-1, pT27w-2, pT27w-3, and pT27w-4, were se-

quenced and their sequence were compared. pT27w-3 has no PCR error and it is defined as *Fusarium sulphureum* lipase nucleotide sequence.

Expression of lipase gene in *Aspergillus oryzae.*

The lipase gene was digested from pT27w-3 with BamH I and Sal I and ligated into the BamH I and XhoI sites in the *Aspergillus* expression cassette pCaHj483 which has *Aspergillus niger* neutral amylase promoter, *Aspergillus nidulans* TPI leader sequences, *Aspergillus niger* glucoamylase terminator and *Aspergillus nidulans* amdS gene as a marker. The resultant plasmid was pNL27w-8.

pNL27w-8 was transformed into *Aspergillus oryzae* BECh-2. The selected transformants were inoculated in 100 ml of MS-9 media and cultivated at 30°C for 1 day. 3 ml of grown cell in MS-9 medium was inoculated to 100 ml of MDU-2BP medium and cultivated at 32°C for 3 days. The supernatant was obtained by centrifugation.

The lipase productivity of selected transformants was determined as LU activity. The productivity of the best transformant TNL27-75 was 130 LU/ml and BECh2 has no lipase activity.

Example 2: Immunological characterization of lipolytic enzyme

A purified lipolytic enzyme sample having the amino acid sequence shown as amino acids 1-319 of SEQ ID NO: 1 was tested by immunodiffusion against a polyclonal antibody raised against the *Fusarium oxysporum* lipase. No immunological cross-reaction was found.

SEQUENCE LISTING

lip3: 5'-carcayggigcigcigcitaytg-3'
lip15: 5'-ccicciariswrtgiccigt-3'
lip10: 5'-ggitgyggigticayiiiggitt-3'
lip17: 5'-ggrtcityiscrtkigtiac-3'
adaptor L: 5'-ctaatacgactcactatagggctcgagcggccgcccgggcaggt=3'
adaptor S: 5'-acctgccc-3'
27N1long: 5'-tggacaaccgttccttgcgca-3'
27C1long: 5'-tacacgtacggtgctcctcgagtgg-3'
27C2: 5'-tatctggcggcggtggcgac-3'
27N(Bam): 5'-cgcggatccatgctcctcctaccactcctctcagcc-3'
27C(Sal): 5'-acgcgtcgacttatgatgaacgattcttatggctatccacatactcct-3'

CLAIMS

1. A lipolytic enzyme which is:

a) a polypeptide encoded by the lipolytic enzyme encoding part of the DNA sequence cloned into a plasmid present in *Escherichia coli* deposit number DSM 13539, or

b) a polypeptide having an amino acid sequence as the mature peptide shown in SEQ ID NO: 1, or which can be obtained therefrom by substitution, deletion, and/or insertion of one or more amino acids;

c) an analogue of the polypeptide defined in (a) or (b) which:

i) has at least 85 % homology with said polypeptide,

ii) is immunologically reactive with an antibody raised against said polypeptide in purified form,

iii) is an allelic variant of said polypeptide,

d) a polypeptide which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with a complementary strand of the nucleic acid sequence of SEQ ID NO: 1 encoding the mature polypeptide or a subsequence thereof having at least 100 nucleotides.

2. The lipolytic enzyme of claim 1 which is native to a strain of *Fusarium*, preferably *F. sulphureum*.

3. A nucleic acid sequence comprising a nucleic acid sequence which encodes the lipolytic enzyme of claim 1 or 2.

4. A nucleic acid sequence which comprises:

a) the partial DNA sequence encoding a mature lipolytic enzyme cloned into a plasmid present in *Escherichia coli* DSM 13539,

b) the partial DNA sequence encoding a mature lipolytic enzyme shown in SEQ ID NO: 1,

c) an analogue of the sequence defined in a) or b) which encodes a lipolytic enzyme and

i) has at least 80 % homology with said DNA sequence, or

ii) hybridizes at high stringency with a complementary strand of said DNA sequence or a subsequence thereof having at least 100 nucleotides,

iii) is an allelic variant thereof, or

d) a complementary strand of a), b) or c).

5. A nucleic acid construct comprising the nucleic acid sequence of claim 3 or 4 operably linked to one or more control sequences capable of directing the expression of the lipolytic enzyme in a suitable expression host.

6. A recombinant expression vector comprising the nucleic acid construct of claim 5, a promoter, and transcriptional and translational stop signals.

7. A recombinant host cell comprising the nucleic acid construct of claim 6.

8. A method for producing a lipolytic enzyme comprising cultivating the host cell of claim 7 under conditions conducive to production of the lipolytic enzyme, and recovering the lipolytic enzyme.

9. A method for preparing a dough or a baked product made from the dough, comprising adding the lipolytic enzyme of claim 1 to the dough.

10. A dough composition comprising the lipolytic enzyme of claim 1.

11. A detergent composition comprising a surfactant and the lipolytic enzyme of claim 1.

SEQUENCE LISTING

<110> Novo Nordisk A/S

<120> Lipase and gene encoding it

<130> NL-27 Fusarium sulphureum lipase

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<141>

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atg ctc ctc cta cca ctc ctc tca gcc gtc act ctc gcg gta gca agt  48
Met Leu Leu Leu Pro Leu Leu Ser Ala Val Thr Leu Ala Val Ala Ser
   -30           -25           -20

cca cta gct tcc gtc gaa gag tac gcc aag tct ctc gaa gac aga g    94
Pro Leu Ala Ser Val Glu Glu Tyr Ala Lys Ser Leu Glu Asp Arg
-15           -10           -5          -1

gtaagcacca aactctcctc catatcatgc tatatactca tcacactccc ag ct gtg 151
                                                  Ala Val
                                                    1

act gtg tct tcg tca gac tac aac aac ttc aag ttc tac atc caa cat  199
Thr Val Ser Ser Ser Asp Tyr Asn Asn Phe Lys Phe Tyr Ile Gln His
       5            10            15
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ggc gcc gca gca tac tgt aac tcc gaa gcc tca gct ggc gca aag atc  247
Gly Ala Ala Ala Tyr Cys Asn Ser Glu Ala Ser Ala Gly Ala Lys Ile
    20              25              30

acc tgc gca agc aac ggt tgt cca acc gtc cag tcc aac ggc gca acc  295
Thr Cys Ala Ser Asn Gly Cys Pro Thr Val Gln Ser Asn Gly Ala Thr
 35              40              45              50

atc gtg gca tcc ttc ct gtaagtcacg cccagtcaca aacatctcat        342
Ile Val Ala Ser Phe Leu
            55

acctcatact tatatgactt cttcag t ggt tcc aag act ggc atc ggc ggt  393
                      Gly Ser Lys Thr Gly Ile Gly Gly
                                  60

tat gtc gca aca gat tca tcc cgc aag gaa atc gtc gtc tcg atc cgt  441
Tyr Val Ala Thr Asp Ser Ser Arg Lys Glu Ile Val Val Ser Ile Arg
 65              70              75              80

gga agc agc aac atc cgc aac tgg ctt aca aac ctc gac ttt gac cag  489
Gly Ser Ser Asn Ile Arg Asn Trp Leu Thr Asn Leu Asp Phe Asp Gln
            85              90              95

tcc gac tgc agc ttg acc tcc ggc tgc ggc gta cac tcg ggc ttc cag  537
Ser Asp Cys Ser Leu Thr Ser Gly Cys Gly Val His Ser Gly Phe Gln
        100             105             110

aac gcc tgg gac gag atc tcg gag aga gcc act gct gct gtg gcc aag  585
Asn Ala Trp Asp Glu Ile Ser Glu Arg Ala Thr Ala Ala Val Ala Lys
    115             120             125

gca cgc aag gca aac tct ggt ttc aag gtc att gct aca ggc cac tcc  633
Ala Arg Lys Ala Asn Ser Gly Phe Lys Val Ile Ala Thr Gly His Ser
130             135             140

ctc ggt ggt gcg gtc gct aca ttg gct gct gcg aat ctg agg gtt ggt  681
Leu Gly Gly Ala Val Ala Thr Leu Ala Ala Ala Asn Leu Arg Val Gly
145             150             155             160

ggc aca ccc gtg gac atc tac acg tac ggt gct cct cga gtg ggc aac  729
Gly Thr Pro Val Asp Ile Tyr Thr Tyr Gly Ala Pro Arg Val Gly Asn
            165             170             175

gcc cag ctt tca gcg ttc atc tcg aac caa gct ggc ggg gaa tat cgc  777
Ala Gln Leu Ser Ala Phe Ile Ser Asn Gln Ala Gly Gly Glu Tyr Arg
        180             185             190

gtt act cac gcc aga gac ccc gtg cct cgt ctg ccc cct ctg gtg ttt  825
Val Thr His Ala Arg Asp Pro Val Pro Arg Leu Pro Pro Leu Val Phe
    195             200             205

gga tac agg cac act tcg ccc gag tac tgg cta tct ggc ggc ggt ggc  873
Gly Tyr Arg His Thr Ser Pro Glu Tyr Trp Leu Ser Gly Gly Gly Gly
210             215             220

gac aag att gat tat acc atc agc gat atc aag gtc tgt gag ggc gcc  921
Asp Lys Ile Asp Tyr Thr Ile Ser Asp Ile Lys Val Cys Glu Gly Ala
225             230             235             240

gct aat ctc cag tgt aac ggt ggc acg ctg ggt ttg gac att gcg gct  969
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Ala Asn Leu Gln Cys Asn Gly Gly Thr Leu Gly Leu Asp Ile Ala Ala
            245             250             255

cat ctg cat tac ttc cag cac act gat gct tgc tcg gcg gga ggc att  1017
His Leu His Tyr Phe Gln His Thr Asp Ala Cys Ser Ala Gly Gly Ile
        260             265             270

tct ttt aga cga tac agg agt gct aag cgt gaa ggt atc gcc aag agg  1065
Ser Phe Arg Arg Tyr Arg Ser Ala Lys Arg Glu Gly Ile Ala Lys Arg
     275             280             285

gct gat atg tcg gat gct gag ctg gag aag aag ctc aac tct tat gtt  1113
Ala Asp Met Ser Asp Ala Glu Leu Glu Lys Lys Leu Asn Ser Tyr Val
   290             295             300

gag atg gat aag gag tat gtg gat agc cat aag aat cgt tca tca taa  1161
Glu Met Asp Lys Glu Tyr Val Asp Ser His Lys Asn Arg Ser Ser
305             310             315             320


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<212> PRT
<213> Fusarium sp.

<400> 2
Met Leu Leu Leu Pro Leu Leu Ser Ala Val Thr Leu Ala Val Ala Ser
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Pro Leu Ala Ser Val Glu Glu Tyr Ala Lys Ser Leu Glu Asp Arg Ala
          20              25              30
Val Thr Val Ser Ser Ser Asp Tyr Asn Asn Phe Lys Phe Tyr Ile Gln
       35              40              45
His Gly Ala Ala Ala Tyr Cys Asn Ser Glu Ala Ser Ala Gly Ala Lys
    50              55              60
Ile Thr Cys Ala Ser Asn Gly Cys Pro Thr Val Gln Ser Asn Gly Ala
 65              70              75              80
Thr Ile Val Ala Ser Phe Leu Gly Ser Lys Thr Gly Ile Gly Gly Tyr
             85              90              95
Val Ala Thr Asp Ser Ser Arg Lys Glu Ile Val Val Ser Ile Arg Gly
         100             105             110
Ser Ser Asn Ile Arg Asn Trp Leu Thr Asn Leu Asp Phe Asp Gln Ser
     115             120             125
Asp Cys Ser Leu Thr Ser Gly Cys Gly Val His Ser Gly Phe Gln Asn
   130             135             140
Ala Trp Asp Glu Ile Ser Glu Arg Ala Thr Ala Ala Val Ala Lys Ala
145             150             155             160
Arg Lys Ala Asn Ser Gly Phe Lys Val Ile Ala Thr Gly His Ser Leu
             165             170             175
Gly Gly Ala Val Ala Thr Leu Ala Ala Ala Asn Leu Arg Val Gly Gly
         180             185             190
Thr Pro Val Asp Ile Tyr Thr Tyr Gly Ala Pro Arg Val Gly Asn Ala
     195             200             205
Gln Leu Ser Ala Phe Ile Ser Asn Gln Ala Gly Gly Glu Tyr Arg Val
   210             215             220
Thr His Ala Arg Asp Pro Val Pro Arg Leu Pro Pro Leu Val Phe Gly
225             230             235             240
Tyr Arg His Thr Ser Pro Glu Tyr Trp Leu Ser Gly Gly Gly Gly Asp
             245             250             255
Lys Ile Asp Tyr Thr Ile Ser Asp Ile Lys Val Cys Glu Gly Ala Ala
         260             265             270
Asn Leu Gln Cys Asn Gly Gly Thr Leu Gly Leu Asp Ile Ala Ala His
```

```
     275                 280                 285
Leu His Tyr Phe Gln His Thr Asp Ala Cys Ser Ala Gly Gly Ile Ser
  290                 295                 300
Phe Arg Arg Tyr Arg Ser Ala Lys Arg Glu Gly Ile Ala Lys Arg Ala
305                 310                 315                 320
Asp Met Ser Asp Ala Glu Leu Glu Lys Lys Leu Asn Ser Tyr Val Glu
            325                 330                 335
Met Asp Lys Glu Tyr Val Asp Ser His Lys Asn Arg Ser Ser
        340                 345                 350
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